

# CLEAN LAKES INC.

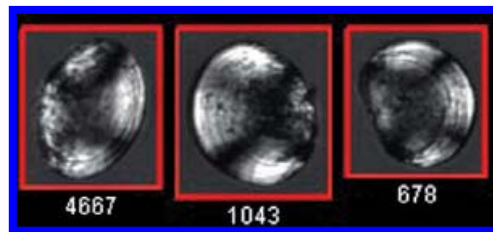
Aquatic Ecosystem Restoration & Maintenance

## Zebra & Quagga Mussel Veliger Detection Services

### ZEBRA AND QUAGGA MUSSEL VELIGER SAMPLE COLLECTION PROCEDURES (PROTOCOL)\*

“WHAT’S IN YOUR WATER?®”

Before collecting plankton samples make arrangements with a laboratory that has the capability to process the samples. Preserve plankton samples per the labs specifications. Zebra and quagga mussels have a planktonic larval life stage (microscopic, free swimming in water column) and are called veligers. Veligers range in size from 70-200 microns (µm).



**SITE LOCATION:** In lake, pond or river systems, samples should be collected from a boat, if possible, at a minimum of three sites in each waterbody. A boat allows the sampling to be independent of land-accessible structures (e.g. docks). Samples should be collected in nearshore and in the open water areas. Sampling may be focused on areas near boat launches and marinas to facilitate boat access (i.e. renting or launching) and reduce field time. In water conveyance systems such as irrigation canals, samples should be collected in the center of the canal (main flow), and can be collected from a bridge or canal crossing.

**SAMPLE FREQUENCY:** Veliger’s can exhibit spatial and temporal patchiness in the water column and high sampling frequency (weekly or biweekly) increases the likelihood of collecting veligers. Veliger production can occur when water temperatures are above 8-12 ° Celsius, so depending upon your location (Southern California Lakes for example can reach water temperatures of 8-12 ° in April, while Northern Idaho Lakes may not reach water temperatures of 8-12 ° until mid June). As such, the optimal time to sample veligers in the Western United States is between April and October, depending on your location and local water temperatures. We suggest that sampling occur a minimum of monthly during the April through October period, or when temperatures are above the 8-12 ° Celsius range. Veliger sampling can be performed anytime during the day but preferably not immediately following a storm event. Storm events can increase water turbidity and hence the time required to process the sample.

## EQUIPMENT & SUPPLIES:

- Plankton net: Simple, conical plankton-tow net, 64 µm pore size, 0.20 m and larger diameter net opening, removable, weighted cod-end piece) (Figure 2). A 64 µm pore size, 0.20 m diameter net opening Plankton net is included in CLI's Veliger Sample Collection Kit. Larger diameter nets are also available, and reduce the tow distance.
- Line for deploying the net: 13 m or about 40 feet long (included in CLI's Veliger Sample Collection Kit)
- Sample container: Polyethylene material, 250 mL volume, screw lid. (included in CLI's Veliger Sample Collection Kit)
- Decontamination materials: Squirt bottle containing 10% solution of household bleach, squirt bottle of tap water. (1 Squirt Bottle included in CLI's Veliger Sample Collection Kit, Bleach Not Included)
- Preservative: 95% ethanol (ETOH) or isopropyl alcohol. (included in CLI's Veliger Sample Collection Kit)
- Field sheets and pen/ pencils (Not Included)
- Global Positioning Satellite unit (GPS) (*recommended, not included in kits*)
- Tweezers or small spatula (*recommended, not included in kits*)
- Boat (*recommended, not included in kits*)
- Multiprobe water quality instrument (e.g. Hydrolab®) (*recommended, not included in kits*)
- Measuring tape or ruler (*optional, not included in kits*)
- Permanent marker (*optional, not included in kits*)

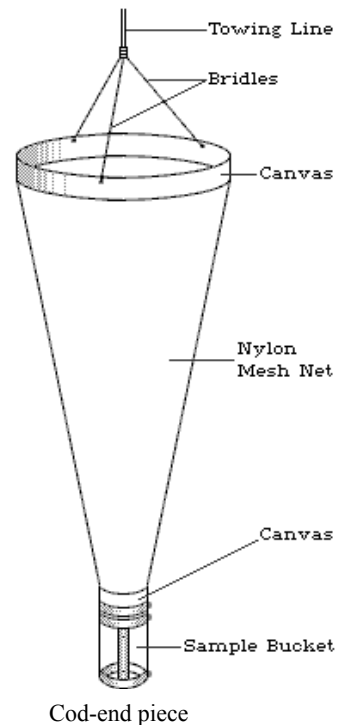


Figure 2: Simple conical plankton net

**PLANKTON NET SAMPLE COLLECTION PROCEDURE:** Collect a recommended minimum of four plankton tows at each site and combine in one sample container. The sample container should be no more than about ¼ full to allow room for preservative. If using CLI's Veliger Sample Collection Kit, the sample containers (bottles) will be pre-filled with preservative. Fill sample containers up to "Max Fill" line. **Do not overfill.** If samples are too large to combine into one bottle and still allow enough volume for the preservative, use a separate sample container for each tow. More than four plankton tows may be collected to increase the likelihood of collecting veligers. Collect each plankton tow in a different area of the site to further increase the likelihood of collecting veligers. Figure 3 depicts plankton collection at a site location.

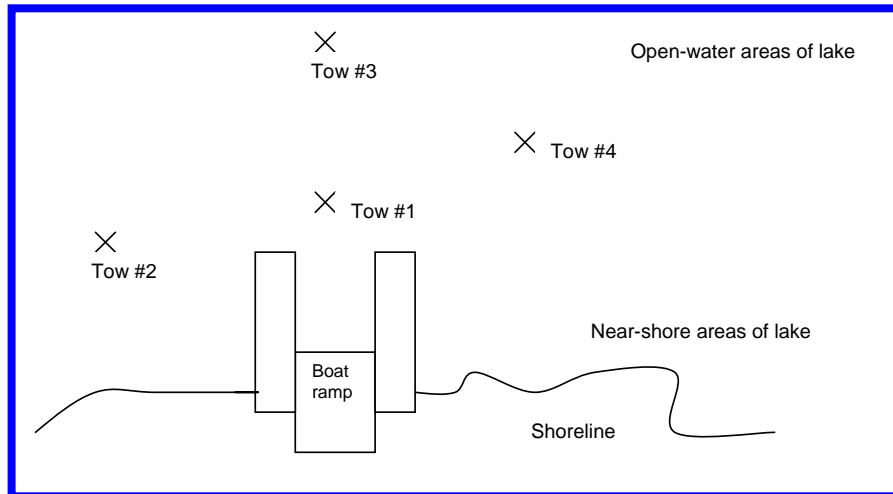


Figure 3: An example of plankton collection at a site location.

### **VERTICAL PLANKTON NET TOW PROCEDURE:**

1. Secure the cod-end piece and check that the line is securely attached to the plankton net. Secure the other end of the line to the boat.
2. Lower the net to depth recommend in Table 1 below for your net diameter, or to 1 m above the sediment. Record the depth the net is lowered into the water.
3. Keep the net at this depth for 60 seconds and then manually retrieve using a hand-over-hand technique at a rate of 0.5 m/ s (1.5 ft/s). Slow and steady retrieval is the key to collecting a good plankton tow.
4. Rinse the net by raising the net so that the cod end of the net is at the water surface. Rinse organisms into the cod end of the net by lowering the net back into the water, keeping the opening above the water surface. Then quickly pull net straight up; this action will move plankton into the cod-end piece. Repeat this procedure several times to ensure that all the organisms inside the net are in the plankton net cod end piece.
5. A squirt bottle, filled with either tap water or water from the lake or river, can be used to squirt down the sides of the net. Spray the outside of the net starting at the mouth to rinse organisms into the plankton net cod end piece.
6. Condense the sample as much as possible before pouring into sample container. Carefully remove the cod-end piece without spilling collected water and plankton. Condense the sample by swirling the cod-end piece. You may need to use tweezers or a spatula to gently clear the mesh netting in the cod-end piece to allow the water to filter through.
7. Lower the cod-end-piece (separated from the plankton net) into the water, keeping the opening above the water surface. Repeat this procedure until the cod-end piece appears clean. Pour the condensed sample into the sample container\*. \*When collecting multiple samples as outlined in Figure 3 above, pour each tows condensed sample into an extra cod-end piece, repeat this procedure for all four samples, and then pour the condensed sample from the extra cod-end piece into the sample container. \*If multiple

tows are required to reach the desired water column tow distance outlined in Table 1 below, pour each tows condensed sample into an extra cod-end piece, repeat this procedure for all multiple samples until the tow distance is met, then pour the condensed sample from the extra cod-end piece into the sample container.

8. It is important to record the number and length of tows so that the quantity of water sampled can be determined. As outlined above (vertical tow procedure 7), condense multiple samples into an extra cod-end piece

**Table 1: Recommended Minimum Vertical Tow Distance**

<b>Plankton Net Diameter (cm)</b>	<b>Water Column Tow Distance (ft)</b>
<b>20</b>	<b>104</b>
<b>50</b>	<b>17</b>

**HORIZONTAL PLANKTON NET TOW PROCEDURE:** Horizontal plankton tows are taken nearshore in depths that are too shallow to collect a vertical tow or in flowing water bodies such as canals. A weight (1 to 2 kg or 2-4 lbs) can be attached to the cod-end piece or to the rope immediately in front of the net opening to keep the net below the water surface if required.

1. Throw the net into the water and allow the net to sink to no more than 1 m above the bottom.
2. For non-flowing, shallow water bodies (shallow areas of lake): Slowly pull the net back to you at a slow and steady rate as described above in the vertical plankton tow.
3. For flowing waterbodies (canals): Leave net in flowing water for the time indicated in Table 2 below depending on flow rate of waterbody.
4. Keep the net off the sediment to avoid both snagging and collecting debris. Note the distance that the net is towed through water and record.
5. Repeat techniques used for vertical plankton tows to concentrate organisms into the cod end of the net.

**Table 2: Recommended Minimum Horizontal Sample Tow Time (seconds)**

<b>Flow Rate (ft/s)</b>	<b>Plankton Net Diameter (20 cm)</b>	<b>Plankton Net Diameter (50 cm)</b>
<b>0.5</b>	<b>209</b>	<b>33</b>
<b>1.0</b>	<b>104</b>	<b>17</b>
<b>1.5</b>	<b>70</b>	<b>11</b>
<b>2.0</b>	<b>52</b>	<b>8</b>
<b>2.5</b>	<b>42</b>	<b>7</b>
<b>3.0</b>	<b>35</b>	<b>6</b>

**REQUIRED METADATA FOR EACH SAMPLE CONTAINER:** See Chain of Custody Form Provided by Clean Lakes, Inc., and or Labels provided in CLI's Veliger Sample Collection Kit

- Site location (GPS coordinates and/ or detailed descriptions)
- Name of waterbody
- Number of tows
- Length of tows (vertical and horizontal)
- Date of collection
- Name of collector
- Client Sample ID

**OPTIONAL DATA:**

- Water temperature and depth(s) of reading (°C)
- Specific conductance (µS/ cm)
- pH

**SAMPLE PRESERVATION PROCEDURE:** Preserve samples in a 70% ETOH (Ethanol (ethyl alcohol)) or isopropyl alcohol solution immediately after collection to ensure sample integrity.

- If using prefilled sample containers provided by Clean Lakes, Inc., add sample to sample containers containing preservative solution to "Max Fill" line. Do NOT overfill.
- Note: If not using Clean Lakes, Inc. sample containers, use the following procedure: To make a 70% solution in the sample container, note the volume of the sample in the container and then add 3 times the volume of 95% ETOH or isopropyl alcohol to the sample. For example, if your sample bottle container contains 1 inch of sample, you would add 3 inches of 95% preservative so that the sample bottle contained 4 inches of combined sample and preservative. This is why it is important to not fill the sample bottle more than ¼ full of sample. A measuring tape or ruler may be placed alongside the sample container to estimate the volumes. ETOH or isopropyl alcohol are the preferred preservatives. **DO NOT USE DENATURED ALCOHOL.**

**EQUIPMENT DECONTAMINATION PROCEDURE:** Field equipment must be decontaminated at the site to prevent transfer of organisms within and between systems. The plankton net, cod-end piece and affiliated rope are decontaminated by spraying with a 10% solution of household bleach (i.e. approximately 0.10 mL of active chlorine per L of water assuming 10% of bleach solution is active chlorine). The 10% bleach solution is carefully poured into a spray bottle and applied to the equipment in a five (5) gallon bucket a minimum of 100 ft from open water. Bleach is corrosive and equipment must be thoroughly rinsed with tap water following decontamination. We suggest using at least 32 oz of clean water to rinse the net following decontamination.

**NOTE:** To prevent cross-contamination and reduce the risk of spreading zebra and quagga mussels, one plankton net, rope, bucket, etc., should be used per site. All sampling gear (including net, rope, wash bottles, buckets, etc) that comes into contact with the water should be soaked in vinegar for 24 hours (an absolute minimum of 4 hours), and rinsed. As an additional recommended precaution, equipment can be sprayed with or soaked in chlorine solution for 5 minutes, and then thoroughly rinsed with clean tap water (the bleach is corrosive so rinse thoroughly with clean water). Dispose of the contaminated rinse water at least 250 ft from any waterbody. The vinegar solution can be reused multiple times. The chlorine solution should be discarded after 24 hours.

- Vinegar Solution: 100% white table vinegar (5% acetic acid solution).
- Chlorine Solution: 10% solution of household bleach (5.25% sodium hypochlorite). To make the bleach solution, add 1.5 cups of household bleach to 1 gal of water. The bleach solution must be fresh (less than 24 hours old). Disassemble net and hang to dry. Routinely inspect the net for damage or wear and repair or replace if necessary.

**SAMPLING LABELING PROCEDURE:** Sample containers must be labeled. Clean Lakes, Inc. has provided labels with the Veliger Sample Collection Kit. The label should contain date, waterbody and detailed location, tow number and length, sampler, and sample ID. This information **MUST** also be recorded on the Clean Lakes, Inc. Chain of Custody form. Below is an example of a label on a sample container.

Date: 01/01/2009
Waterbody: Columbia River
Location: Chinook Boat Landing Boat Ramp
N 44.3221374 W 122.4552241
Tow Data: 4 tows: 25ft, 25ft, 16ft, 12ft
Sampler: John Doe
Sample ID: 00001

**SAMPLE HANDLING AND CHAIN OF CUSTODY:** Samples preserved with ETOH may be stored in a cool, dry place a maximum of three months prior to analysis. Avoid placing samples in direct sunlight or freezing conditions. Samples that cannot be preserved immediately after collection should be placed on ice until preservative can be added. Do not wait more than 3 hours to preserve samples.

ETOH (Ethanol (ethyl alcohol)) and isopropyl alcohol are Class 3 flammable liquids and there are restrictions regarding its transport. ETOH and isopropyl alcohol can only be transported on the ground/surface. Do not fly in an airplane with ETOH or isopropyl alcohol. Keep preserved samples in a plastic container such as a bin or cooler in the back of the car while in transit. If CLI's Veliger Sample Collection Kit has been purchased, please follow the "Shipping Instructions" insert provided by Clean Lakes, Inc. If the Veliger Sample Collection Kit was not purchased, ship or mail preserved samples to Clean Lakes, Inc., or another laboratory via ground or surface mail using United States Postal Service (USPS) and/or Federal Express according to the protocols below, which allow exemptions for training and certification.

USPS Protocols for mailing ETOH:

1. Samples must be in plastic containers with a screw lid. There can be one or multiple containers but the total volume of the entire package cannot exceed 473 mL. Secure screw lids with tape.
2. Place all containers into a sealable plastic bag (e.g. Zip Lock) and then place this bag into another sealable plastic bag.
3. Place sealed bags and sample containers into box and add cushioning material such as grocery bags or scrap paper. Seal this box with clear packing tape. The box does NOT need to be a specific type of box so long as it is sturdy.
4. Place this box into another box and add cushioning material as needed. The outer box does NOT need to be a specific type of box either, so long as it is sturdy. Seal box with clear packing tape.
5. **Include a complete return address on the package and also label the side of box with the information shown to the right:**

Surface Mail Only Consumer Commodity ORM-D Flashpoint = 55.6°F
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FedEx Protocols for mailing ethanol:

1. Samples must be in plastic containers with a screw lid. The volume in each container cannot exceed 30 mL. Secure screw lids with tape.
2. A maximum of 16 containers per box. The total volume in all the containers can NOT exceed 500 mL.
3. Place all containers into a sealable plastic bag (e.g. Zip Lock) and then place this bag into another sealable plastic bag.
4. Place sealed bags and sample containers into a box and add cushioning material such as plastic grocery bags or scrap paper. Seal this box with clear packing tape. The box does NOT need to be a specific type of box so long as it is sturdy.
5. Place this box into another box and add cushioning material as needed. The outer box does NOT need to be a specific type of box so long as it is sturdy. Seal box with clear packing tape.
6. **Include a complete return address. The label that is placed on address side of box is shown to the right:**

This package conforms to 49 CFR 173.4
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For more information, contact Leif Elgethun, Clean Lakes, Inc. Attn: Veliger Detection Division, 1770 West State Street #125, Boise, ID 83702 (cell: 208-301-2293), (Fax: 888-330-8602), or (Email: [lelgethun@cleanlake.com](mailto:lelgethun@cleanlake.com)), or contact one of our offices below.

**Pacific Northwest: Coeur D'Alene, Idaho: Phone: 208-665-1475, Fax: 208-665-1479**  
**San Francisco Bay Area: Martinez, California: Phone: 925-957-1905, Fax: 925-957-1906**  
**Santa Monica Bay Area: Westlake Village, California: Phone: 818-889-8691, Fax: 818-889-8693**

*\*The information in this protocol was adapted from the Portland State University, Center for Lakes and Reservoirs "Veliger Collection Protocol", the California Department of Water Resources "Zebra and Quagga Mussel Veliger Sampling Protocol for the State Water Project", April 29, 2008, and CLI's internal protocols.*